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=> s inhibit?(2W)(ribonuclease or RNAse)

             0 INHIBIT?
             0 RIBONUCLEASE
             0 RNASE
L1           0 INHIBIT?(2W)(RIBONUCLEASE OR RNASE)
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```
=> s ribonuclease

L2            0 RIBONUCLEASE

=> file caplus

COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
                                                         ENTRY    SESSION
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FILE COVERS 1967 - 1 Mar 1999  VOL 130 ISS 10
FILE LAST UPDATED: 1 Mar 1999  (19990301/ED)

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  This file supports REG1stRY for direct browsing and searching of
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=> s ribonuclease

L3         7493 RIBONUCLEASE

=> s inhbit?(2W)(ribonuclease or RNAse)

            225 INHBIT?
           7493 RIBONUCLEASE
          26792 RNASE
L4            0 INHBIT?(2W)(RIBONUCLEASE OR RNASE)

=> s inhibit?(2W)(ribonuclease or RNASE)

        1138104 INHIBIT?
           7493 RIBONUCLEASE
          26792 RNASE
L5          851 INHIBIT?(2W)(RIBONUCLEASE OR RNASE)

=> d L5 851


L5   ANSWER 851 OF 851  CAPLUS  COPYRIGHT 1999 ACS
AN   1967:8319  CAPLUS
DN   66:8319
TI   Ribonucleic acids of the endoplasmic reticulum of animal cells
AU   Rodionova, N. P.; Shapot, V. S.
CS   Acad. Med. Sci. U.S.S.R., Moscow, USSR
SO   Biochim. Biophys. Acta (1966), 129(1), 206-9
     CODEN: BBACAQ
DT   Journal
LA   English

=> s chelator or chelating

           6580 CHELATOR
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          30092 CHELATING
L6        35022 CHELATOR OR CHELATING

=> s L6 and L5

L7            5 L6 AND L5

=> d L7 1-5


L7   ANSWER 1 OF 5  CAPLUS  COPYRIGHT 1999 ACS
AN   1995:970399  CAPLUS
DN   124:25038
TI   Factors affecting flow cytometric detection of apoptotic nuclei by DNA
     analysis
AU   Elstein, Kenneth H.; Thomas, David J.; Zucker, Robert M.
CS   National Health and Environmental Effects Research Laboratory, U.S.
     Environmental Protection Agency, Research Triangle Park, NC, USA
SO   Cytometry (1995), Volume Date 1995, 21(2), 170-6
     CODEN: CYTODQ; ISSN: 0196-4763
DT   Journal
LA   English

L7   ANSWER 2 OF 5  CAPLUS  COPYRIGHT 1999 ACS
AN   1994:186872  CAPLUS
DN   120:186872
TI   The catalytic properties of the reverse transcriptase of the lentivirus
     equine infectious anemia virus
AU   Rubinek, Tami; Loya, Shoshana; Shaharabany, Miriam; Hughes, Stephen H.;
     Clark, Patrick K.; Hizi, Amnon
CS   Sackler Sch. Med., Tel Aviv Univ., Israel
SO   Eur. J. Biochem. (1994), 219(3), 977-83
     CODEN: EJBCAI; ISSN: 0014-2956
DT   Journal
LA   English

L7   ANSWER 3 OF 5  CAPLUS  COPYRIGHT 1999 ACS
AN   1992:16803  CAPLUS
DN   116:16803
TI   Aluminum interrupts the formation of alkaline-ribonuclease-inhibitor
     complex from bovine brain
AU   Cho, Sung Woo; Kim, Geum Yi
CS   Coll. Med., Univ. Ulsan, Seoul, 138-040, S. Korea
SO   Eur. J. Biochem. (1991), 202(1), 107-11
     CODEN: EJBCAI; ISSN: 0014-2956
DT   Journal
LA   English

L7   ANSWER 4 OF 5  CAPLUS  COPYRIGHT 1999 ACS
AN   1979:470753  CAPLUS
DN   91:70753
TI   Reverse transcriptase-associated RNase H.  III.  Reverse
     transcriptase-associated ribonuclease H does not require zinc for
     catalysis
AU   Modak, Mukund J.; Srivastava, Arun
CS   Memorial Sloan-Kettering Cancer Cent., New York, NY, 10021, USA
SO   J. Biol. Chem. (1979), 254(11), 4756-9
     CODEN: JBCHA3; ISSN: 0021-9258
DT   Journal
LA   English

L7   ANSWER 5 OF 5  CAPLUS  COPYRIGHT 1999 ACS
AN   1971:9771  CAPLUS
DN   74:9771
TI   Extracellular nuclease activity of Micrococcus sodonensis.  III.  Kinetic
     studies and control of production
```

AU Berry, Sheila A.; Campbell, James N.
CS Dep. Microbiol., Univ. Alberta, Edmonton, Alberta, Can.
SO Biochim. Biophys. Acta (1970), 220(2), 256-68
CODEN: BBACAQ
DT Journal
LA English

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=> s ribonuclease(W)inhibitor#

L8 470 RIBONUCLEASE(W) INHIBITOR#

=> s EDTA and L8

L9 8 EDTA AND L8

=> d L9 1-8

L9 ANSWER 1 OF 8 MEDLINE
AN 92037631 MEDLINE
DN 92037631
TI Aluminum interrupts the formation of alkaline-**ribonuclease-inhibitor** complex from bovine brain.
AU Cho S W; Kim G Y
CS Department of Biochemistry, College of Medicine, University of Ulsan, Seoul, Korea.
SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (1991 Nov 15) 202 (1) 107-11.
Journal code: EMZ. ISSN: 0014-2956.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199202

L9 ANSWER 2 OF 8 MEDLINE
AN 81026444 MEDLINE
DN 81026444
TI Isolation of giant silk fibroin polysomes and fibroin mRNP particles using
a novel **ribonuclease inhibitor**, hydroxystilbamidine.
AU Lizardi P M
NC GM-22865 (NIGMS)
SO JOURNAL OF CELL BIOLOGY, (1980 Oct) 87 (1) 292-6.

Journal code: HMV. ISSN: 0021-9525.
CY Country: United States
DT Document Type: Journal; Article; (JOURNAL ARTICLE)
LA Language: English
FS File Segment: Priority Journals
EM Entry Month: 198102

L9 ANSWER 3 OF 8 CAPLUS COPYRIGHT 1999 ACS
AN Accession Number: 1998:129464 CAPLUS
DN Document Number: 128:202352
TI Title: Endogenous **ribonuclease inhibitors** of mammals, cDNAs encoding them, and their uses
IN Inventor Name: Chatterjee, Deb K.; Shandilya, Harini
PA Patent Assignee: Life Technologies, Inc., USA; Chatterjee, Deb K.; Shandilya, Harini
SO Source: PCT Int. Appl., 78 pp.
CODEN: PIXXD2
DT Document Type: Patent
LA Language: English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9806845	A1	19980219	WO 97-US14254	19970814
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9740648	A1	19980306	AU 97-40648	19970814
PRAI US 96-24057		19960816		
US 97-794546		19970203		
US 97-795395		19970204		
US 97-910731		19970813		
WO 97-US14254		19970814		

L9 ANSWER 4 OF 8 CAPLUS COPYRIGHT 1999 ACS
AN Accession Number: 1992:16803 CAPLUS
DN Document Number: 116:16803
TI Title: Aluminum interrupts the formation of alkaline-**ribonuclease-inhibitor** complex from bovine brain
AU Author Name: Cho, Sung Woo; Kim, Geum Yi
CS Corporate Source: Coll. Med., Univ. Ulsan, Seoul, 138-040, S. Korea
SO Source: Eur. J. Biochem. (1991), 202(1), 107-11
CODEN: EJBCAI; ISSN: 0014-2956
DT Document Type: Journal
LA Language: English

L9 ANSWER 5 OF 8 CAPLUS COPYRIGHT 1999 ACS
AN Accession Number: 1991:242030 CAPLUS
DN Document Number: 114:242030
TI Title: Molecular cloning and expression of human placental **ribonuclease inhibitor** cDNA
IN Inventor Name: Lewis, Martin Kendall; Shultz, John William
PA Patent Assignee: Promega Corp., USA
SO Source: PCT Int. Appl., 42 pp.
CODEN: PIXXD2
DT Document Type: Patent
LA Language: English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9012881	A1	19901101	WO 90-US2122	19900418
W: AU, JP				
RW: AT, BE, CH, DE, DK, ES, FR, GB, IT, LU, NL, SE				

	AU 9055377	A1	19901116	AU 90-55377	19900418
	AU 646803	B2	19940310		
	EP 422217	A1	19910417	EP 90-908084	19900418
	EP 422217	B1	19980128		
	R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, LU, NL, SE				
	JP 03505677	T2	19911212	JP 90-506833	19900418
	AT 162853	E	19980215	AT 90-908084	19900418
	ES 2111537	T3	19980316	ES 90-908084	19900418
	US 5552302	A	19960903	US 94-282151	19940727
PRAI	US 89-342362	19890424			
	US 90-510881	19900418			
	WO 90-US2122	19900418			
	US 92-856863	19920324			

L9 ANSWER 6 OF 8 CAPLUS COPYRIGHT 1999 ACS
AN 1981:510314 CAPLUS
DN 95:110314
TI Effect of human placental **ribonuclease inhibitor** in cell-free ribosomal RNA synthesis
AU Eichler, Duane C.; Tatar, Todd F.; Lasater, Linda S.
CS Coll. Med., Univ. South Florida, Tampa, FL, 33612, USA
SO Biochem. Biophys. Res. Commun. (1981), 101(2), 396-403
CODEN: BBRCA9; ISSN: 0006-291X
DT Journal
LA English

L9 ANSWER 7 OF 8 CAPLUS COPYRIGHT 1999 ACS
AN 1980:634445 CAPLUS
DN 93:234445
TI Isolation of giant silk fibroin polysomes and fibroin mRNP particles using a novel **ribonuclease inhibitor**, hydroxystilbamidine
AU Lizardi, Paul M.
CS Dep. Cell Biol., Rockefeller Univ., NY, 10021, USA
SO J. Cell Biol. (1980), 87(1), 292-6
CODEN: JCLBA3; ISSN: 0021-9525
DT Journal
LA English

L9 ANSWER 8 OF 8 CAPLUS COPYRIGHT 1999 ACS
AN 1967:496893 CAPLUS
DN 67:96893
TI Purification of **ribonuclease inhibitor** from pig cerebral cortex
AU Takahashi, Yasuo; Mase, Keikichi; Suzuki, Y.
CS Niigata Univ. Sch. Med., Niigata, Japan
SO Experientia (1967), 23(7), 525-6
CODEN: EXPEAM
DT Journal
LA English

=> log y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	10.20	35.26

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  *               W E L C O M E   T O   T H E                        *
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=> s hydroxyquinoline (P)phenol

          3086 HYDROXYQUINOLINE
         97043 PHENOL
L1         238 HYDROXYQUINOLINE (P)PHENOL

=> s RNA or DNA

         17776 RNA
         30040 DNA
L2       31193 RNA OR DNA

=> s L1 and L2

L3          58 L1 AND L2

=> d L3 1-58


1.  5,866,410, Feb. 2, 1999, Cloning of the biosynthetic pathway for
chlortetracycline and tetracycline formation and cosmids useful therein;
Michael J. Ryan, et al., 435/320.1 [IMAGE AVAILABLE]

2.  5,846,531, Dec. 8, 1998, Marine mela gene; Ronald M. Weiner, et al.,
424/94.4; 435/189 [IMAGE AVAILABLE]

3.  5,837,505, Nov. 17, 1998, Melanin production from transformed
escherichia coli; Guy della-Cioppa, et al., 435/128, 193, 244, 252.33;
536/23.2, 23.4 [IMAGE AVAILABLE]

4.  5,817,631, Oct. 6, 1998, Therapeutic uses of melanin; David L.
Berliner, et al., 514/21; 424/94.4, 195.11; 514/64, 567 [IMAGE AVAILABLE]

5.  5,814,495, Sep. 29, 1998, Melanin production by streptomyces; Guy
della-Cioppa, et al., 435/120; 424/60; 435/191, 252.35, 253.5 [IMAGE
AVAILABLE]

6.  5,807,527, Sep. 15, 1998, Solid medium and method for DNA
storage; Leigh Alexander Burgoyne, 435/5, 6, 7.1, 7.2, 7.9, 91.2;
536/24.3, 24.32, 24.33 [IMAGE AVAILABLE]

7.  5,776,968, Jul. 7, 1998, Therapeutic uses of melanin; David L.
Berliner, et al., 514/414, 12, 415 [IMAGE AVAILABLE]

8.  5,756,126, May 26, 1998, Dry solid medium for storage and analysis of
genetic material; Leigh Alexander Burgoyne, 424/488; 422/55, 56, 57;
435/4, 5, 6, 7.1, 7.2, 7.9, 91.2, 174, 183, 970 [IMAGE AVAILABLE]

9.  5,743,477, Apr. 28, 1998, Insecticidal proteins and method for plant
```

protection; Terence A. Walsh, et al., 424/94.6; 435/198 [IMAGE AVAILABLE]

10. 5,703,051, Dec. 30, 1997, Therapeutic uses of melanin; David L Berliner, et al., 514/21; 424/94.4, 195.11; 514/63, 567 [IMAGE AVAILABLE]

11. 5,663,048, Sep. 2, 1997, Y-chromosome specific polynucleotide probes for prenatal sexing; Robert J. Winkfein, et al., 435/6, 91.2; 536/24.3 [IMAGE AVAILABLE]

12. 5,656,596, Aug. 12, 1997, Method of treating lesions in a nervous system; Denis Monard, et al., 514/12; 435/69.4; 514/2; 530/399; 536/23.5, 23.51; 930/120 [IMAGE AVAILABLE]

13. 5,631,151, May 20, 1997, Melanin production by transformed organisms; Guy della-Cioppa, et al., 435/133, 108, 189; 536/23.2 [IMAGE AVAILABLE]

14. 5,591,605, Jan. 7, 1997, Plant structural gene expression; Timothy C. Hall, et al., 800/294; 435/69.1, 320.1, 414, 415, 416, 419; 536/23.6, 24.1; 800/298, 300, 301, 302, 317, 317.3, 322 [IMAGE AVAILABLE]

15. 5,589,385, Dec. 31, 1996, Cloning of the biosynthetic pathway for chlortetracycline and tetracycline formation and cosmids useful therein; Michael J. Ryan, et al., 435/252.35, 252.3, 252.31, 252.32, 252.33, 320.1; 536/23.2, 23.7 [IMAGE AVAILABLE]

16. 5,532,246, Jul. 2, 1996, Use of 1,3-oxathiolane nucleoside analogues in the treatment of hepatitis B; Bernard Belleau, deceased, et al., 514/274 [IMAGE AVAILABLE]

17. 5,529,909, Jun. 25, 1996, Tyrosinase-activator protein fusion enzyme; Guy della-Cioppa, et al., 435/69.7, 189, 252.3, 252.33, 252.35, 320.1; 536/23.2, 23.4 [IMAGE AVAILABLE]

18. 5,504,200, Apr. 2, 1996, Plant gene expression; Timothy C. Hall, et al., 800/298; 435/69.1, 70.1, 252.2, 252.3, 252.33, 320.1, 419; 536/23.6; 800/300, 301, 302, 317.3, 322 [IMAGE AVAILABLE]

19. 5,496,562, Mar. 5, 1996, Solid medium and method for **DNA** storage; Leigh A. Burgoyne, 424/488, 443, 464 [IMAGE AVAILABLE]

20. 5,486,520, Jan. 23, 1996, 1,3-oxathiolanes useful in the treatment of hepatitis; Bernard Belleau, deceased, et al., 514/274, 49 [IMAGE AVAILABLE]

21. 5,413,915, May 9, 1995, Method and sensor for detecting toxic chemical exposure effects and metabolic activation of carcinogenic chemical agents; George D. Case, et al., 435/25; 422/56; 435/287.9, 288.7, 317.1 [IMAGE AVAILABLE]

22. RE 34,875, Mar. 14, 1995, Method of selecting recombinant **DNA**-containing streptomyces; Virginia A. Birmingham, et al., 435/475, 252.3, 252.35, 320.1, 476, 486; 536/23.1, 23.2, 23.7 [IMAGE AVAILABLE]

23. 5,357,636, Oct. 25, 1994, Flexible protective medical gloves and methods for their use; Karl P. Dresdner, Jr., et al., 2/161.7, 167, 168, 169 [IMAGE AVAILABLE]

24. 5,310,678, May 10, 1994, Newcastle disease virus gene clones; Richard W. Bingham, et al., 435/252.3, 69.3, 235.1, 252.31, 252.33, 252.35, 254.11; 536/23.72 [IMAGE AVAILABLE]

25. 5,268,290, Dec. 7, 1993, Process for producing neuraminidase; Mamoru Hasegawa, et al., 435/201, 69.1, 200, 252.35, 320.1; 536/23.2 [IMAGE AVAILABLE]

26. 5,245,026, Sep. 14, 1993, Metal containing 8-hydroxyquinoline chelating agents; David K. Johnson, et al., 540/3, 470, 474; 546/2, 178, 180 [IMAGE AVAILABLE]

27. 5,233,044, Aug. 3, 1993, Active esters for solid phase peptide synthesis; Derek Hudson, 548/110, 368.1, 371.1 [IMAGE AVAILABLE]

28. 5,198,360, Mar. 30, 1993, **DNA** sequence conferring a plaque inhibition phenotype; Margaret M. Ballou, et al., 435/252.3, 252.35, 320.1; 536/23.72 [IMAGE AVAILABLE]

29. 5,187,080, Feb. 16, 1993, **DNA** encoding an antigenic protein derived from Eimeria tenella and vaccines for prevention of coccidiosis caused by Eimeria tenella; William H. Andrews, et al., 435/69.3; 424/191.1, 267.1; 435/69.1, 91.41, 235.1, 252.3, 252.33, 320.1; 530/300, 350, 388.6; 536/23.4, 23.7 [IMAGE AVAILABLE]

30. 5,165,925, Nov. 24, 1992, Vaccine for immunizing fish against infectious pancreatic necrosis virus; Jo-ann C. Leong, 424/186.1, 204.1, 817; 435/69.3; 536/23.72 [IMAGE AVAILABLE]

31. 5,130,250, Jul. 14, 1992, Molecular cloning and expression of neutral protease genes; Alan H. Deutch, et al., 435/252.33, 68.1, 91.41, 221, 320.1; 530/350; 536/23.2 [IMAGE AVAILABLE]

32. 5,102,796, Apr. 7, 1992, Plant structural gene expression; Timothy C. Hall, et al., 435/252.2, 252.3, 320.1; 536/23.2, 23.6, 23.7, 24.1 [IMAGE AVAILABLE]

33. 5,047,345, Sep. 10, 1991, Composition for isolating and purifying nucleic acid and improved method using same; David A. DeBonville, et al., 435/270, 6, 259, 803; 536/25.41, 25.42 [IMAGE AVAILABLE]

34. 5,039,667, Aug. 13, 1991, Antiviral therapy for hepatitis B with 2',3'-dideoxypurine nucleosides; David L. J. Tyrrell, et al., 514/45; 424/43, 433, 436, 464; 514/46; 536/27.14, 27.6, 27.61, 27.8, 27.81 [IMAGE AVAILABLE]

35. 5,032,520, Jul. 16, 1991, **DNA** sequences encoding infectious bronchitis virus spike protein; Matthew M. Binns, et al., 435/364, 69.1, 70.1, 91.41, 91.51, 235.1, 236, 320.1; 536/23.72, 24.1 [IMAGE AVAILABLE]

36. 5,032,501, Jul. 16, 1991, **DNA** probes to vntr loci; Eric C. B. Milner, 435/6; 536/24.3, 24.31 [IMAGE AVAILABLE]

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(FILE 'USPAT' ENTERED AT 08:47:57 ON 01 MAR 1999)
L1 238 S HYDROXYQUINOLINE (P)PHENOL
L2 31193 S RNA OR DNA
L3 58 S L1 AND L2

=> d L3 52

52. 4,833,239, May 23, 1989, Method for the isolation and purification of **DNA** molecules; David A. DeBonville, et al., 536/25.41 [IMAGE AVAILABLE]

=> d ab

US PAT NO: 5,866,410 [IMAGE AVAILABLE] L3: 1 of 58

ABSTRACT:
The present invention describes a purified and isolated nucleic acid molecule which encodes for the biosynthetic pathway of tetracycline, chlortetracycline or an analogue thereof. The invention relates to the isolation and cloning of the nucleic acid molecule in an isolated fragment from Streptomyces aureofaciens and the expression of the biosynthetic gene in a heterologous host such as Streptomyces lividans.

=> d kwic

US PAT NO: 5,866,410 [IMAGE AVAILABLE] L3: 1 of 58

SUMMARY:

BSUM(4)

The . . . which can be protoplasted and regenerated. More importantly, protoplasts later proved to be an ideal substrate for transformation by plasmid **DNA**, thus creating the opportunity to do recombinant **DNA** experiments in these organisms (Bibb et al., 1978). The isolation of genes for several antibiotic resistances, such as thiostrepton, viomycin. . .

SUMMARY:

BSUM(7)

Another . . . for antibiotic biosynthesis were physically linked to the resistance determinant(s) for that same antibiotic in the producing organism. Thus, a **DNA** fragment from Streptomyces ariseus conferring streptomycin resistance was shown to be contiguous with **DNA** that complemented biosynthetic blocks (Distler et al., 1985). The same situation was seen in Streptomyces fradiae where biosynthetic genes had been identified by probing a cosmid library for homology to a mixed-base oligonucleotide constructed to represent the **DNA** sequence for the amino-terminus of the final enzyme in the tylosin biosynthetic pathway (Fishman et al., 1989). A previously cloned tylosin resistance gene (tlrB) was shown to be contained within this region of **DNA**, which complemented nine classes of blocked mutants (Baltz et al., 1988). In the cases of puromycin (Vara et al., 1988). . . resistance gene in the heterologous host Streptomyces lividans allowed subsequent identification of antibiotic biosynthetic genes located on the same cloned **DNA** fragment.

SUMMARY:

BSUM(8)

The . . . by hybridization to both a previously cloned resistance determinant (Butler et al., 1989) and an oligonucleotide synthesized to represent the **DNA** sequence corresponding to the partially elucidated amino acid sequence of the biosynthetic enzyme anhydrotetracycline oxygenase (Binnie et al., 1989). The. . .

SUMMARY:

BSUM(11)

Two . . . in the same S. lividans host, tetracenomycin was produced. Bifunctional clones isolated from an E. coli library of Streptomyces peucetius **DNA** by hybridization to actI and actIII probes of S. coelicolor were shown to direct the synthesis of pigmented antibiotic when. . .

SUMMARY:

BSUM(14)

The present invention is the first instance wherein the single **DNA** gene cluster related to the entire biosynthetic pathway for producing tetracycline and chlortetracycline is isolated and utilized.

DRAWING DESC:

DRWD(4)

FIG. . . . 3. The vector portion is represented by double line. The TC/CTC biosynthetic region is shown as a single line. The **DNA** cloned from S. aureofaciens is 31.9 kb; the vector is 11.1 kb. The vector regions denoted are pIBI-24 (hatched), thiostrepton-resistance. . . two EcoRI sites marked with a (+) are vector-derived and flank the Sau3A-BglII junction which demarcates vector and S. aureofaciens **DNA**.

DRAWING DESC:

DRWD(5)

FIG. 3 shows the restriction endonuclease map for S. aureofaciens **DNA** which is cloned in LP.sup.2 127 and LP.sup.2 128. The 31.9 kb of **DNA** cloned in LP.sup.2 127 and LP.sup.2 128 is shown in linear form. The map is drawn so as to include. . .

DRAWING DESC:

DRWD(6)

FIGS. 4A-4L show the total **DNA** sequence from the cosmid clones designated LP.sup.2 127 and LP.sup.2 128 (this sequence is also set forth in Sequence I.D.. . . obtained using the dideoxy chain termination method (Sanger et al., Proc. Natl. Acad. Sci. USA, 74:5463-5467, 1977). The S. aureofaciens **DNA** carried in the cosmid clones is fragmented either by digestion with appropriate restriction endonuclease or by sonication. The smaller pieces. . . et al., Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989). The **DNA** sequencing is carried out at elevated temperatures using Tag **DNA** polymerase employing fluorescently-labeled primers using materials and methods supplied by the manufacturer (Applied Biosystems, Foster City, Calif.). The data are collected using a Model 370A/373A **DNA** sequencing system (Applied Biosystems, Foster City, Calif.). Compilation of the data, generation of overlapping sequences and the overall analysis of this **DNA** sequence information are carried out using the collection of standard computer programs contained within the Genetics Computer Group package (Devereaux. . .

DETDESC:

DETD(2)

The . . . host such as Streptomyces lividans. In particular, the present invention concerns the purified and isolated nucleic acid molecule, e.g., a **DNA** gene cluster, coding for the biosynthetic pathway for producing the antibiotics or an analogue thereof.

DETDESC:

DETD(5)

In . . . The nucleotide sequence of the nucleic acid molecule is shown in FIG. 4. Desirably, the nucleic acid molecule is a **DNA** gene cluster isolated from Streptomyces aureofaciens, or an antibiotic-producing mutant thereof, and expressed in a suitable heterologous host, such as. . .

DETDESC:

DETD(6)

The present invention further includes the **DNA** sequences which hybridize under standard or stringent conditions to the sequence of the nucleic acid molecule isolated from the microbial. . .

DETDESC:

DETD(7)

Additionally, . . . Together, the plasmids comprise an efficient cosmid vector which allows for the cloning and packaging of large, contiguous pieces of **DNA**. It is contemplated that these plasmids described herein may be employed for cloning large **DNA** from any source.

DETDESC:

DETD(8)

For . . . biosynthetic genes, a screen of a recombinant S. lividans library for a clone expressing tetracycline-resistance is utilized. The S. aureofaciens **DNA** inserts in the recombinant cosmids which comprise the library are large since the constraints of the in vitro lambda packaging system demands cosmid molecules with **DNA** inserts of 25-40 Kb to yield a viable transducing phage particle. When tetracycline resistant clones are selected from among this. . .

DETDESC:

DETD(9)

The method for isolating the **DNA** involves lysozyme digestion of cells in an osmotic buffer, followed by gentle lysis, protein extraction and enrichment for, and concentration of, high molecular weight **DNA**. Although the method described is efficient, those skilled in the art will recognize that a variety of alternative procedures may. . .

DETDESC:

DETD(10)

The source of total **DNA** used in the examples is Streptomyces aureofaciens ATCC 13899 but the invention is not limited to this particular source. A. . .

DETDESC:

DETD(11)

A partial digestion of S. aureofaciens **DNA** with restriction endonuclease Sau3A to generate large **DNA** fragments in the desired 35-kilobase size range with ends homologous to those of the arms of the bifunctional cosmid vector. . . products by agarose gel electrophoresis. Those skilled in the art will recognize alternative library construction and recovery methods for cloned **DNA** of interest. The present invention is not limited to the use of Escherichia coli and the size selection imposed by. . .

DETDESC:

DETD(12)

The steps that follow in the examples involve in vitro packaging of the ligation products of cosmid arms and size fractionated **DNA**, transduction to E. coli X2819T, collection of the population of transductants and isolation of **DNA** from them to give a cosmid library. The methods used are described, but the invention is not limited by those. . .

DETDESC:

DETD(13)

Subsequent steps in the examples describe introduction of the pooled cosmid **DNA** preparation into Streptomvces lividans, creation of a cell library and subsequent screening of such a library for transformants of S.. . .

DETDESC:

DETD(14)

Next, recovery of recombinant plasmid by isolating plasmid **DNA** from the tetracycline-resistant S. lividans followed by in vitro packaging of said **DNA** and transduction into E. coli is obtained. Plasmid **DNA** isolated from such transductants is structurally characterized by restriction enzyme mapping analysis; and the two plasmids isolated in the example,. . . LP.sup.2 127 and LP.sup.2 128, are shown to possess equivalent structures. Those skilled in the art will recognize that similar **DNA** regions cloned from alternative organisms could show polymorphism in the arrangement of restriction sites, but that a sufficiently large **DNA** fragment conferring tetracycline-resistance would be expected to confer the properties described hereinbelow.

DETDESC:

DETD(16)

Finally, . . . LP.sup.2 127 transformant of S. lividans produce tetracycline and chlortetracycline under conditions where the same products are isolated from the **DNA** source organism Streptomyces aureofaciens ATCC 13899. On the other hand, a S. lividans transformant containing only plasmid vector with no inserted **DNA** shows no antibiotic production.

DETDESC:

DETD(19)

PREPARATION OF STREPTOMYCES AUREOFACIENS TOTAL **DNA**

DETDESC:

DETD(22)

Once lysis is complete, 20 mL of equilibrated **phenol** (50 g **phenol**+6.5 mL of 100 mM NaCl, 10 mM Tris pH8, 1 mM EDTA pH8+0.05 g 8-**hydroxyquinoline**) is added, the preparation gently shaken and then spun in a table top centrifuge at 1500 .times.g for 30 minutes. The aqueous top layer is collected and re-extracted as above; the spent **phenol** from the first extraction is back-extracted with 20 mL 10 mM Tris pH7.4, 1 mM EDTA pH 8 (TE). The. . . pH 5 is added to each and 10 mL of cold ethanol layered on top of the viscous solution. The **DNA** is gently spooled onto a glass rod, rinsed twice in cold ethanol and dissolved in 8 mL TE overnight at 4.degree. C. An A.sub.260 spectrophotometric reading is taken as an estimate of total nucleic acids present (predominantly **DNA**).

DETDESC:

DETD(24)

PARTIAL DIGESTION AND SIZE ENRICHMENT OF S. AUREOFACIENS **DNA**

DETDESC:

DETD(25)

A partial digestion condition that yields Sau3A digestion products of S. aureofaciens **DNA** in the range of 35 kilobases (Kb) is determined empirically. A series of reaction tubes containing .about.25 .mu.g **DNA** contained in 300 .mu.L of reaction buffer consisting of 100 mM NaCl, 10 mM Tris pH7.4 10 mM MgCl.sub.2 are. . . and restriction endonuclease Sau3A (New England Biolabs) added to give final concentrations of 0.5, 0.1, 0.05, 0.01, 0.005 enzyme units/.mu.g **DNA**. The reactions are incubated at 37.degree. C. for 60 minutes, then placed at 65.degree. C. for 20 minutes and finally. . . ice. Twenty .mu.L is removed and loaded to 0.5% agarose gel for size comparison to fragments of known length (lambda **DNA** digested with HindIII, XhoI and undigested). The **DNA** in the remaining volume is precipitated by the sequential additions of 50 .mu.L 3M ammonium acetate and 1 mL ethanol, followed by chilling at -20.degree. C. The precipitated **DNA** is then pelleted by centrifugation at 8800 .times.g, redissolved in 300 .mu.L 0.3M ammonium acetate, similarly precipitated, pelleted, rinsed with. . . the ethidum bromide stained agarose gel which is electrophoresed overnight at 1 volt/cm, reveals that digestion with 0.05 units Sau3A/.mu.g **DNA** gives digestion products largely in the desired 35 Kb size range.

DETDESC:

DETD(29)

Plasmid A is digested with Asp718 and then desphosphorylated with calf intestine alkaline phosphatase (CIAP). The **DNA** then is extracted with chlorpane and chloroform, precipitated with ethanol and vacuum dried. The **DNA** is then resuspended and digested with BglII. Plasmid B is digested with SalI and subsequently treated with CIAP. After chlorpane extraction, ethanol precipitation and vacuum drying, the **DNA** is resuspended and digested with BglII.

DETDESC:

DETD(33)

LIGATION OF COSMID ARMS TO SAU3A DIGESTED GENOMIC **DNA** AND IN VITRO PACKAGING

DETDESC:

DETD(34)

The Sau3A digested and size "inspected" genomic fragments of S. aureofaciens **DNA** are joined to cosmid arms via in vitro ligation. Four .mu.L Sau3A digested S. aureofaciens **DNA**, corresponding to .about.8 .mu.g, are combined with 1 .mu.g each of cosmid arms 1 and 2 in a 10 .mu.L. . . 66 mM Tris pH7.4, 10 mM MgCl.sub.2, 1 mM ATP, 10 mM dithiothreitol and 40 units (cohesive end unit) T4 **DNA** ligase (New England Biolabs). The ligation mixture is incubated at 11.degree. C. for 18 hours then subjected to an in vitro packaging reaction by adding the entire 10 .mu.L reaction to a Packagene.sup.R lambda **DNA** packaging system extract (Promega Biotec). After a 2 hour incubation at room

temperature, 500 .mu.L phage dilution buffer (PDB) (100. . .

DETDESC:

DETD(37)

The . . . transduced into Escherichia coli X2819T (R. Curtiss), with the objective of obtaining thousands of transductants from which a pooled plasmid **DNA** preparation, or bifunctional cosmid library, can be obtained. To this end, 0.3 mL of an overnight culture of X2819T is. . .

DETDESC:

DETD(38)

Each . . . The aqueous solution is brought to 6 mL with TE; 1 mL 3M ammonium acetate is added and the plasmid **DNA** precipitated with 18 mL of ethanol. After chilling at -20.degree. C. the **DNA** is pelleted by centrifugation at 3400 .times.g for 30 minutes. A second precipitation is similarly performed, then the **DNA** is rinsed with ethanol, vacuum dired, dissolved in 1 mL TE and the **DNA** concentration is determined spectrophotometrically.

DETDESC:

DETD(41)

The . . . spinning at 3400 .times.g for 10 minutes and then resuspended in the residual volume. Approximately 10 .mu.g of cosmid library **DNA** is added to each, followed by the addition of 0.5 ML of 25% PEG1000 (1 g PEG1000 (Sigma) dissolved in. . .

DETDESC:

DETD(45)

The . . . or L-leucine) containing 100 .mu.g of tetracycline/mL. The growth obtained after two days is then processed for isolation of plasmid **DNA** as previously described except that all volumes employed are four times that of the previous example. The final **DNA** precipitate is dissolved in 1 mL TE.

DETDESC:

DETD(46)

A 10 .mu.L portion of the plasmid **DNA** isolated from S. lividans transformant LL535 is subjected to an in vitro packaging reaction and subsequently transduced to E. coli. . . 500 mL portions of 20-10-5 broth containing 100 .mu.g of amplicillin/mL. After incubation at 30.degree. C., 200 rpm overnight plasmid **DNA** is isolated again as previously described. The isolated plasmid is designated LP.sup.2 127; the estimated size of the plasmid is. . .

DETDESC:

DETD(47)

A . . . the vector portion, such as EcoRI, EcoRV or HindIII. Restriction endonuclease digestions are performed by combining 1-2 .mu.g of plasmid **DNA** 4 .mu.l of a 10.times. solution of salts that are

optimal for the restriction endonuclease being employed and approximately 5-40. . .

DETDESC:

DETD(48)

Digestion . . . of LP.sup.2 127 is shown in FIG. 2. A more detailed restriction endonuclease map for the 31.9kb at S. aureofaciens **DNA** cloned in LP.sup.2 127 is shown in FIG. 3.

DETDESC:

DETD(51)

The . . . containing 10 .mu.g of thiostrepton/mL and 100 .mu.g of tetracycline/mL. After five days incubation at 28.degree. C., 200 rpm, plasmid **DNA** is prepared by a minipreparation procedure, which is similar to previously described plasmid isolation procedures up to the isopropanol precipitation. . . the nucleic acid pellet is dissolved in 1 mL TE and extracted with an equal volume of chlorpane (500 g **phenol** and 0.5 g 8-**hydroxyquinoline** equilibrated in a buffer containing 100 mM NaCl, 1 mM EDTA pH8, 10 mM sodium acetate pH 6, plus 500. . .

DETDESC:

DETD(59)

A . . . produce CTC and TC on agar and in broth fermentation, whereas S. lividans containing a plasmid cloning vector without inserted **DNA** does not yield a tetracycline antibiotic. LP.sup.2 128 transformed into S. lividans directs the synthesis of an antiobiotic with acitivity. . .

DETDESC:

DETD(63)

The . . . LL531 are grown on Bennetts agar containing 25 .mu.g of thiostrepton/mL; S. aureofaciens ATCC 13899, the source of the cloned **DNA** in LP.sup.2 2127 and LP.sup.2 128, is plated on Bennetts agar without drug. After five days of growth at 30.degree.. . .

DETDESC:

DETD(66)

Thiostrepton-resistant . . . whereas LP.sup.2 63 transformants do not, thereby indicating that the ability to produce antibiotic is associated with the S. aureofaciens **DNA** present in LP.sup.2 127 and LP.sup.2 128.

DETDESC:

DETD(72)
3. Bibb M. J., J. M. Ward, and D. A. Hopwood. 1978. Transformation of plasmid **DNA** into Streptomyces protoplasts at high frequency. Nature (London) 274:398-400.

DETDESC:

DETD(77)

7. . . . H. -F. Lin, C. L. Kuo. H. -L. Tsai and J. F. -Y. Tsai. 1988. Cloning and expression of a **DNA** sequence conferring cephamycin C production. Bio/Technology 6:1222-1224.

DETDESC:

DETD(85)
15. Hohn, B. and J. Collins. A small cosmid for efficient cloning of large **DNA** fragments. Gene 11:291-298.

DETDESC:

DETD(92)
22. Larson, J. L. and C. L. Hershberger. 1986. The minimal replicon of a streptomycete plasmid produces ultrahigh level of plasmid **DNA**. Plasmid 15:199-209.

DETDESC:

DETD(101)
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